

ANALYTICAL METHOD AND APPARATUS FOR BLOOD USING NEAR INFRARED SPECTROSCOPY

BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The present invention relates to an analytical method and apparatus using a near infrared (NIR) spectroscopy which determine chemical components and physicochemical characteristics (hereinafter referred to as "object characteristics") of (the) blood such as red blood cells, hematocrit, hemoglobin, total protein, total cholesterol
10 and blood sugar.

2. Description of the Prior Art

In the prior art, the collected blood has been separated into blood plasma and red blood cells by centrifugal separation, and the blood plasma which is the supernatant liquid has been analyzed by an automatic blood analyzer and the like to determine the
15 object characteristics of the blood.

Simple analytical methods which are disclosed in the National Publication of the Translated Version of PCT Application Nos. Hei 5-506171 and Hei 7-503863 are also known.

20 According to these methods, it is possible to determine the components of the blood, e.g. glucose concentration in the blood, by applying near infrared light to a finger or an ear to measure a reflectance spectrum without collecting blood from the human body.

25 The method for separating the blood into blood plasma and red blood cells to automatically analyze the blood plasma that is the supernatant liquid is lacking in promptness. Such analysis not only requires the use of many reagents, but also a great deal of skill. It is therefore a problem as an analytical method of the blood carried out on site by an unskilled operator.

30 On the other hand, the methods that have been disclosed in the National Publication of the Translated Version of PCT Application Nos. Hei 5-506171 and Hei 7-503863 are simple, but the blood is not measured directly. Accordingly, much noise is generated and there is a problem in measurement accuracy.

SUMMARY OF THE INVENTION



It is therefore an object of the present invention is to provide an analytical method and apparatus which can simply and precisely carry out an analysis of the object characteristics of the blood.

To attain the above-mentioned object, according to the present invention, an analytical method of ^{analyzing} blood is provided, which comprises the steps of: ^(a) applying near infrared light to the blood in a translucent blood collection tube or bag from the outside through the blood collection tube or bag; ^(b) detecting, ^{and} diffusely reflected light ^{sample} or diffusely transmitted and reflected light from the blood in the blood collection tube or bag using an optical sensor to measure a near infrared absorption spectrum of the blood, and ^{replacing} the measured value with a value obtained from a calibration equation which has been ^{determined} in advance from a spectrum measured using the same method as above, thereby determining the object characteristics of the blood.

According to conventional near infrared spectroscopy, near infrared light in a wavelength range of 1100nm - 2500nm has been used. It ^{has} ^{been} therefore necessary to prepare a special crystal sample cell with an optical path length of 0.1 - 2mm. Operations such as cleaning, drying and filling of the ^{blood} sample are therefore troublesome and require time. Further, because of the narrow optical path length, non-uniformity of the sample and existence of impurities have a great influence on measured results. However, even in the case of near infrared light, if near infrared light in a short wavelength range of 700nm - 1100nm is used, its penetration force is 10 to 100 times as large as that in a long wavelength range (1100nm - 2500nm). Accordingly, when the near infrared light in the short wavelength range is used, the optical path length can be maintained at a level of 1 - 2cm and the blood analysis can be carried out with the blood contained in the ^{ordinary} blood collection tube or bag. ^{receptacle such as a}

When the near infrared light is applied to an object (the blood), only a specified wavelength light is absorbed in proportion to the number of molecules out of various molecules contained in the object. The wavelength of the light absorbed varies with the structure of the molecule (kind of molecule). The blood contains various kinds of components and generates a complicated absorption phenomenon in which absorptions overlap. The near infrared absorption spectrum is obtained by plotting the absorbance (i.e. the degree to which the light is absorbed) against wavelengths.

To conduct quantitative analysis by using this near infrared absorption spectrum, a regression equation (a calibration equation) that relates a value of the object

characteristics (the concentration or the characteristic value) to spectrum data is required. Usually, the spectrum of a sample of which the value of the object characteristics ^{is} ^{me} known is measured. Based on the spectrum data and the object characteristics value, the calibration equation can be made by a chemometrics technique
5 such as multiple linear regression (MLR), principal component regression (PCR) and PLS regression (PLS).

Further, to attain the above-mentioned object, according to the present invention, the apparatus for blood analysis is provided, which comprises a block provided with a housing portion for a translucent blood collection ^{receptacle} (tube or bag), a near
10 infrared apparatus provided with a spectroscope for dispersing near infrared light and an optical sensor for detecting the near infrared light from a source of light or ^{from a light source} a light from a sample, light conduction means for conducting the near infrared light emitted from the light source or the spectroscope to the blood collection ^{receptacle} (tube or bag) within the housing portion and for conducting, directly or through the spectroscope, ^{at least one of} diffusely reflected light, ^{sample} diffusely transmitted light or diffusely transmitted and reflected light from the blood, within the blood collection ^{receptacle} (tube or bag) to the optical sensor, and control means for outputting a measurement command of a spectrum ^{of the blood sample} to the near infrared apparatus and for ^{modifying} replacing the measured value with a value obtained from ^{measured} a calibration equation which has been ^{determined} made in advance, thereby computing object characteristics (chemical
15 components or physiochemical characteristics) of the blood ^{to be measured} ^{sample}.

As the light source, it is preferable to use a metal halide lamp (a white light source) such as a tungsten halogen lamp because of its high intensity. A diode array is considered preferable as the optical sensor because it is easy for the diode array to be compacted and there is also some possibility that the diode array will be widely used
25 from now on.

Further, when the monochromatic near infrared light is used as the light source, it is preferable to use a silicon detector or a lead sulfide detector that is commonly used as the optical sensor.

As the light conducting means, it is preferable to use an optical fiber (a single
30 fiber) or an optical fiber bundle (a bundle of optical fibers).

It is also possible to realize a high precision measurement if the block is provided with a temperature control means for stabilizing the blood ^{sample} within the blood collection ^{receptacle} (tube or bag) at a predetermined temperature.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and advantages of the present invention will become more apparent from the following description when taken in conjunction
5 with the accompanying drawings.

Fig. 1 is a general view of one example of an apparatus for carrying out an analytical method for blood according to the present invention;

Fig. 2 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes a reference material;

10 Fig. 3 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes the blood;

Fig. 4 is a ^{chart}view showing a near infrared absorption spectrum of arterial blood and venous blood measured by a diffuse transmittance method;

15 Fig. 5 is a ^{chart}view showing the near infrared absorption spectrum of venous blood measured by a diffuse reflectance method;

Fig. 6 is a ^{obtained}view showing an example of measurement of hemoglobin (Hb) of the blood by a near infrared spectroscopy; and

20 Fig. 7 is a ^{obtained}view showing an example of measurement of hematocrit of the blood by a near infrared spectroscopy.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A preferred embodiment of the present invention will now be described with reference to the accompanying drawings. Fig. 1 is a general view showing one example of an apparatus for carrying out an analytical method of blood according to the present
25 invention. Fig. 2 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes a reference material and Fig. 3 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes the blood.

30 An analytical apparatus for carrying out an analytical method of blood of the present invention is, as shown in Fig. 1, provided with a dispersive type of near infrared apparatus 1 and a computer 2 to control it. The near infrared apparatus 1 is provided therein with a spectroscope for dispersing a near infrared light and an optical sensor for detecting the near infrared light from a white light from a source of light. An aluminum-made block 3 is attached to the near infrared apparatus 1.

This block 3 is formed with a housing portion 5 which can contain a blood collection tube 4 therein. The upper surface of the housing portion 5 is open and a cap 6 is arranged to prevent light from entering this open portion.

One end of an optical fiber 7 is connected to the spectroscope which is provided within the near infrared apparatus 1 and the other end thereof faces the inside of the housing portion 5. One end of an optical fiber 8 is connected to the optical sensor provided within the near infrared apparatus 1 and the other end thereof faces a position opposite to the other end of the optical fiber 7 on the inside of the housing portion 5. The optical fibers 7 and 8 are protected by a bellows tube 9.

Provided under the block 3 is a heating apparatus 10 such as a panel heater for stabilizing the blood within the blood collection tube 4 at a predetermined temperature. A controller 11 is arranged to control this heating apparatus 10.

A spectrum measurement procedure for a blood sample using the analytical apparatus stated above will now be explained.

First, a ceramic plate 12 which is a reference material for spectrum measurement is set within the housing portion 5 of the aluminum block 3 and a light shielding cap is set to cover the housing portion 5. The computer 2 is then operated to measure the transmitted light intensity of the ceramic plate 12. Namely, the monochromatic near infrared light in a range of 700nm - 1100nm from slits of the near infrared apparatus 1 is applied to the ceramic plate 12 through the optical fiber 7. The light diffusely transmitted through the ceramic plate 12 is detected through the optical fiber 8 by the optical sensor provided within the near infrared apparatus 1.

The near infrared apparatus 1 can scan a predetermined wavelength range in about 0.5 seconds. The near infrared apparatus usually repeats the scan about 50 times and the measurements are averaged to obtain the transmitted light intensity of the ceramic plate 12 at each wavelength.

Next, in place of the ceramic plate 12, the blood collection tube 4 containing a blood sample adjusted at a predetermined temperature by a water bath and the like is inserted into the housing portion 5. The transmitted light intensity of the blood samples is then measured using the same procedure as above.

The absorbance as shown by a formula (1) is computed by the computer 2 and a so-called near infrared absorption spectrum in which the absorbance has been plotted against wavelengths is displayed on the screen of the computer 2.

$$A(\lambda) = \log \{ E_r(\lambda) / E_s(\lambda) \} \quad (1)$$

wherein, $A(\lambda)$: absorbance at the wavelength of λ nm
 $E_r(\lambda)$: intensity of light transmitted through the ceramic plate at the wavelength of λ nm
 $E_s(\lambda)$: intensity of light transmitted through the blood sample at the wavelength of λ nm

Fig. 4 shows the near infrared absorption spectra of arterial blood and venous blood of a goat measured in a diffuse transmittance method and Fig. 5 shows the near infrared absorption spectrum of the venous blood of the goat measured in a diffuse reflectance method. An absorption band of water of 970nm is observed in each spectrum. In the venous blood, an absorption band of 760nm due to reduced hemoglobin can be seen. It is not possible to clearly observe the absorption band due to the object characteristics, but the information for the object characteristics is also included in the same spectra. Thus, to extract the information for each object characteristic from the spectrum data, a calibration equation that relates each object characteristic to the spectrum data is necessary.

The calibration equation for measuring the object characteristics of the blood sample, e.g. the hemoglobin concentration, will be described below.

(A) Prepare at least 100 specimens of ^{the} blood sample having a wide range of the hemoglobin concentration.

(B) Collect ^{one of} the blood sample ^{specimens} in the blood collection tube 4 and adjust the temperature of the blood collection tube at a predetermined temperature by a water bath. Measure the near infrared absorption spectrum of the blood sample according to the method stated above. Repeat this operation for the number of ^{sample} specimens.

(C) Analyze the hemoglobin concentration of each blood sample by ^{the} conventional chemical method.

(D) Input the analyzed hemoglobin concentration in the corresponding spectrum data file.

(E) Divide the spectrum data with the hemoglobin concentration into two data sets for calibration and validation. ^{respectively}

(F) Carry out MSC treatment, derivative treatment and the like on the spectrum data of calibration set as a pretreatment for the near infrared absorption spectrum as

occasion demands.

(G) Using the pretreated spectrum data of calibration set, prepare a plurality of relational equations (regression equations) which can be candidates for the calibration equation by a chemometrics technique such as MLR, PCR and PLS.

- 5 (H) Using the spectrum data of validation set, which has not been used for calibration, evaluate the performance of the relational equations made in the preceding paragraph by standard error of prediction (SEP). Adopt the equation with the smallest SEP as the calibration equation at the time of the routine analysis.

10 Table 1 shows a result of the PLS using the second derivative spectra of the blood measured by the diffuse reflectance method.

Table 1

Results of PLS regression using the second derivative spectra of the blood

Object Characteristics	F	R	SEC	SEP	Bias
Hemoglobin (%)	3	0.99	0.26	0.28	0.00
Hematocrit (%)	3	0.99	0.81	0.86	0.04
Oxygen (%)	4	0.90	1.95	2.34	-0.03

20 F: Number of factors used in the calibration equation

R: Multiple correlation coefficient

SEC: Standard error of calibration

SEP: Standard error of prediction

25 Bias: Difference between a mean value of the values according to a conventional method and a mean value of NIR values

The correlation coefficient between an actual value of hemoglobin (Hb) analyzed by a known chemical method and NIR measured value is 0.99 and the SEC is 0.26%. The SEP is 0.28%. In the case of the routine analysis, measurement is made with the error of this SEP value.

30 In the case of the routine analysis, the following calibration equation is used to measure, for example, the hemoglobin (Hb).

$$\text{Hb (\%)} = F1 \cdot q1 + F2 \cdot q2 + F3 \cdot q3 + F4 \cdot q4 \quad (2)$$

where, $F_i = \sum A(\lambda) \cdot W_i(\lambda)$

F_i : i^{th} factor (where, $i = 1 - 4$)

$A(\lambda)$: original spectrum of the blood (absorbance at λ nm)

$W_i(\lambda)$: i^{th} loading weight (where, $i = 1 - 4$)

q_i : i^{th} regression coefficient (where, $i = 1 - 4$)

The concentration of hemoglobin (Hb) can be computed using the formula (2) from absorbance in each wavelength because the regression coefficient q_i and the loading weight $W_i(\lambda)$ are the constants to be determined according to the object characteristics. Quantitative analysis can also be carried out for hematocrit, oxygen, and other object characteristics using the same method as above.

(In the) ^{For purpose of} routine on-site analysis and the like, an analytical procedure for the hemoglobin concentration of the blood is as follows. However, ^{involving} the above-mentioned calibration equation shall be stored in the near infrared apparatus 1 or the computer 2.

(A) The electrical supply of the near infrared apparatus 1 is switched on. After the near infrared apparatus 1 is stabilized, a reference spectrum is measured using the reference ceramic plate 12.

(B) ^{The} blood sample contained in the blood collection tube 4 is adjusted to a predetermined temperature by the water bath.

(C) The blood collection tube 4 containing the blood sample of which the temperature has been adjusted is loaded in the housing portion 5 of the aluminum block 3. The computer 2 is operated to allow the near infrared apparatus 1 to measure the spectrum.

(D) After the spectrum measurement is completed, the computer 2 computes the hemoglobin concentration based on the calibration equation stored therein and the spectrum obtained, and displays the hemoglobin concentration on the screen.

(E) Repeat the operations (B) - (D) for the number of samples. Time required for the operations of the steps (C) and (D) is about 30 seconds.

The relationship ^S between ^S an actual value ^S analyzed by the known chemical method and the ^{measured} NIR value ^S in the event that the contents of hemoglobin (Hb) and hematocrit of the blood are analyzed by a routine analysis is shown in Figs. 6 and 7, respectively.

In the above-mentioned embodiment, the object characteristics of the blood are measured using the diffuse transmittance method, but it is also possible to use the diffuse reflectance method or the transfectance (transmittance + reflectance) method. In such a case, the arrangement of the optical fiber of course differs from the above.

- 5 As described above, according to the present invention, it is possible to measure the object characteristic value⁵ of ^{sampled} (the) blood, with the blood contained in ^{sampled} (the) an ordinary translucent container such as the blood collection tube and bag. Accordingly, it is also possible to get the information about the main components of the blood ^{sampled} on-site ^{for} where the blood collection. With this construction, nutritional diagnosis, medical examination or
- 10 the like is realized on the spot.

Samples
are
collected

¶ Although there has been described what is the present embodiment of the invention, it will be understood that variations and modifications may be made thereto without departing from the gist, spirit or essence of the invention. The scope of the invention is indicated by the appended claims, rather than by the foregoing description of the present embodiment.

What is claimed is:

1. An analytical method of ^{analyzing} blood using near infrared spectroscopy comprising the steps of:
 - (a) applying light ^{to the blood in a translucent blood collection tube or bag from the outside} through the ^{receptacle} blood collection tube or bag ^{to a sample of the blood contained in the receptacle}; and
 - (b) detecting ^{at least one of} diffusely reflected light ^{and} diffusely transmitted light ^{or} diffusely transmitted and reflected light ^{from the blood in the blood collection tube or bag by an optical sensor to measure a near infrared absorption spectrum of the blood; and}
 - (c) ^{modifying} replacing the measured ^{determined} value with a value obtained from ^{spectrum using} a calibration equation which has been ^{made} in advance from a spectrum measured using the same method as above, thereby determining ^{an} object characteristics ^{sample} of the blood.
2. The analytical method of blood according to claim 1, wherein the wavelength of near infrared light applied to the blood in the blood collection tube or bag is 700nm - 1100nm.
- 15 3. The analytical method of ^{analyzing} blood according to claim 1, wherein the calibration equation is ^{determined using} made by a chemometrics technique [such as multiple linear regression (MLR), principal component regression (PCR) and PLS regression].
4. An analytical apparatus ^{for analyzing} of blood comprising:
 - a block provided with a housing portion for a translucent blood collection tube or bag; ^{receptacle}
 - a near infrared apparatus provided with a spectroscope for dispersing near infrared light from ^{a light source or from a sample of blood contained in the receptacle} and an optical sensor for detecting the near infrared light from a source of light or a light from a sample;
- 25 light conduction means for conducting the near infrared light emitted from the light source or the spectroscope to the blood collection tube or bag within the housing portion and for conducting, directly or through the spectroscope, ^{at least one of} diffusely reflected light ^{and} diffusely transmitted light ^{or} diffusely transmitted and reflected light from the blood within the blood collection tube or bag to the optical sensor; and
- (^{sample}) control means for outputting a ^{measured} measurement command of a spectrum to the near infrared apparatus and for ^{modifying} replacing the measured spectrum with a value obtained from a calibration equation which has been ^{determined} made in advance, thereby computing ^{an} object characteristics ^{sample} of the blood ^{to be measured}.
- 30 5. The analytical apparatus of blood according to claim 4, wherein a white light

Steps (a) and (b) relative to blood specimens with known object characteristics

of the blood sample

for analyzing

source [such as a tungsten halogen lamp] is used as the light source, and a diode array is used as the optical sensor.

6. The analytical apparatus ^{for analyzing} of blood according to claim 4, wherein ^{the} monochromatic near infrared light is used as the light source, and a silicon detector or a lead sulfide detector is used as the optical sensor.
7. The analytical apparatus ^{for analyzing} of blood according to claim 4, wherein the light conduction means ^{compr. of} an optical fiber or an optical fiber bundle.
8. The analytical apparatus ^{for analyzing} of blood according to claim 4, wherein the block is provided with a temperature control means for stabilizing the blood ^{sample} within the blood collection ^{receptacle} [tube or bag] at a predetermined temperature.

ABSTRACT OF THE DISCLOSURE

A method of analyzing blood using a near infrared

- 5 [First] monochromatic near infrared light in a wavelength range of 700nm - 1100nm from the slit of the near infrared apparatus [1] is applied to a ceramic plate through ^{an} [the] optical fiber [7] to measure a transmitted light intensity of the ceramic plate, which is a reference material for spectrum measurement. Next, in place of the ceramic plate, a blood collection tube [4] containing a blood sample of which [the temperature] has been (adjusted) ^{stabilized} at a predetermined temperature by a water bath [and the like is inserted into the housing portion 3]. ~~replace the near infrared light applied thereto.~~ The transmitted light intensity of the blood sample is thus measured using the same procedure as above. A so-called near infrared absorption spectrum in which absorbance has been plotted against wavelengths is displayed on the screen of the computer 2. Information ^{the blood} about object characteristics is extracted from the spectrum data using a calibration equation.
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apparatus,
in which

obtained and information

pre determined
using blood
specimens
with different, known
object characteristics.